

P.C. 2108.91

PATENT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re application of

LAWRENCE A. JOHNSON

Method to Preselect the
Sex of Offspring

Serial No. 07/692,958

Filed April 26, 1991

Group Art Unit 188
Examiners J. Witz &
D. W. Robinson

[2108DP]

DECLARATION UNDER 37 CFR 1.132

Dan Pinkel deposes and states:

1. That he considers himself to be an expert in the art of preparing mammalian sperm for flow cytometry as evidenced by the attached curriculum vitae;

2. That he has read and is familiar with the following publications authored by Johnson et al.:

■ "Flow Sorting of X and Y Chromosome-Bearing Spermatozoa Into Two Populations," *Gamete Research* 16:1-9 (1987);

■ "Flow Cytometry of X and Y Chromosome-Bearing Sperm for DNA Using an Improved Preparation Method and Staining With Hoechst 33342," *Gamete Research* 17:203-212 (1987);

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■"Flow Sorting of X and Y Chromosome-Bearing Mammalian Sperm: Activation and Pronuclear Development of Sorted Bull, Boar, and Ram Sperm Microinjected Into Hamster Oocytes," *Gamete Research* 21:1-9 (1988);

3. That in his opinion the various treatments preparatory to staining the sperm cells in each of the publications referred to in (2) would in every case cause the tails and midsections of the sperm to become severed from the sperm heads and that the plasma and acrosomal membranes surrounding the resultant sperm heads would thereby be disrupted;
4. That the treatments referred to in (3) would render the sperm cells completely nonviable and would facilitate absorption of stain such as Hoechst 33342 by the disrupted sperm;

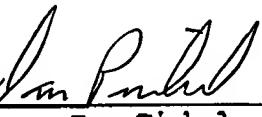
5. That, absent any knowledge he has of the subject matter of the above-identified application, he would have expected that any extended incubation of viable sperm at temperatures within the range of 30-39° C would be detrimental to the ultimate viability of the sperm and would not be a recommended procedure;

6. That in his opinion incubation at room temperature for a period of 3-5 hours would tend to decrease viability of sperm that was intended for use in artificial insemination;

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7. That it is his understanding that treatments accorded sperm for Artificial Insemination always include cooling as soon as practicable after ejaculation to begin the slowing of sperm metabolism and that in his experience in the area of sperm measurement, one would never consider incubating sperm at 30-39° C for extended time periods, because it is contrary to the maintenance of sperm viability; and
8. That all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed 12-26, 1991.



Dan Pinkel

CURRICULUM VITAE

DANIEL PINKEL

PERSONAL

BIRTH: Cleveland, Ohio, March 25, 1944

ADDRESS:

Residence: 31 Manzanita Court
Walnut Creek, CA 94595
(tel. 510-932-0990)

Business: Department of Laboratory Medicine
University of California, San Francisco
MCB 230
San Francisco, California 94143-0808
(tel. 415-476-3659)

EDUCATION:

University of Michigan, B.A. (Physics), 1966.
University of California, San Diego, Ph.D. (Physics), 1974.

POSITIONS:

NSF Graduate Fellow and Research Assistant, University of California, San Diego, 1966-1974.

Assistant Research Physicist, University of California, San Diego, 1/1975-6/1975.

NIH Postdoctoral Fellow, University of California, Los Angeles, 9/1975-2/1977.

Biophysicist, Lawrence Livermore Laboratory, University of California, Livermore, California, 2/1977 to 7/91.

Associate Professor in Residence, University of California, San Francisco, 7/91 to present.

RESEARCH:

Electron Spin Resonance in Metals, Flow Cytometry, In Situ Hybridization, Automated Microscopy

HONORS: Phi Beta Kappa, Sigma XI, Phi Kappa Phi.

SOCIETIES:

American Association for the Advancement of Science
International Society for Analytical Cytology (founding member)
American Society of Human Genetics
Sigma Xi
Federation of American Scientists

PROFESSIONAL ACTIVITIES:

NIH special study sections/ site visits

National Research Council of Canada grant reviewer

Dutch Cancer Society grant reviewer

Manuscript reviewer -- Science, PNAS, Genomics, American Journal of Human Genetics, Cytometry, Chromosoma, Human Genetics, Genes Chromosomes and Cancer, Cancer Genetics and Cytogenetics

Teaching lab workshops for fluorescence in situ hybridization--

Los Alamos, New Mexico 1986

Paris, France 1987;

Tucson, Arizona 1989

Montevideo, Uruguay 1989

Baltimore, Maryland 1990 (key faculty)

Session chairman

Cytogenetics; American Society of Human Genetics Annual Meeting November 11-15, 1989.

Poster Symposium on Fluorescence in situ Hybridization; 8th International Society of Human Genetics, Oct. 6-11, 1991

Workshop on in situ hybridization. 83rd Annual Meeting of the American Association of Cancer Research, San Diego CA, May 20 - 23, 1992.

Meeting organization

Fluorescence in situ Hybridization: Molecular Probes in Tumor Diagnosis, Houston, Texas, Oct. 27-29, 1991 (co-organizer with Dr. M. Andreef, M. D. Anderson Cancer Center)

Advisory panels

Institute of Medicine, National Academy of Sciences -- Research Briefing: Advances in Understanding Genetic Changes in Cancer, Impact on Diagnosis and Treatment Decisions in the 1990s. March 5-6, 1991

INVITED LECTURES:

U. S. Department of Agriculture Beltsville Symposia in Agricultural Research 11: Research Instrumentation for the 21st Century, May 4 - 8, 1986.

International Society of Analytical Cytology plenary lecture
Breckenridge CO, September 4 - 8, 1988.

American Association of Clinical Chemistry 40th National Meeting, New Orleans LA. July 24-28, 1988.

Medical Applications of Lasers and Optics (SPIE conference), Los Angeles CA, January 15 - 20, 1989.

American Society for Cell Biology Education Committee Workshop -- In situ hybridization. San Francisco CA. January 29, 1989.

Third International Workshop on Chromosomes in Solid Tumors. Tucson AZ. February 26-March 1, 1989.

21st Annual Oak Ridge Conference on Advanced Analytical Concepts for the Clinical Laboratory. Special topic -- Recombinant DNA Technology for the Clinical Chemist. San Diego CA. April 13-14, 1989.

1989 Annual Course in Flow Cytometry. Los Alamos NM. May 21-26, 1989.

Fifth International Conference on Environmental Mutagens. Cleveland OH, July 10 - 15, 1989.

Clinical Applications of Flow Cytometry Fourth Annual Meeting. Keynote address. Charleston SC, September 13 - 16, 1989.

Leukemia Research Fund International Summit Workshop on CML. Cape Cod, October 5 - 8, 1989.

MARHGN meeting on in situ hybridization. Baltimore MD. September 17 - 18, 1990.

The Instrument and the Cell: Methods of Cytometric Analysis. Seventh National Meeting of the Italian Cytometry Group. Pisa, Italy. November 5 - 7, 1990.

29th Annual American Cytogenetics Conference. Tahoe City, CA. February 20 - 23, 1991.

Fourth International Workshop on Chromosomes in Solid Tumors. Tucson AZ. February 24 - 26, 1991.

Congress of the Japan Cytometry Society. June 20 - 21, 1991.

MRC Edinburgh, Scotland. August 19, 1991.

International Society of Analytical Cytology. Bergen, Norway. August 25 - 30, 1991.

International Symposium on Molecular Cytogenetics, February 5 - 8, 1992.

Gordon Conference on Molecular Cytogenetics, March 2 - 6, 1992.

83rd Annual Meeting of the American Association of Cancer Research, San Diego CA, May 20 - 23, 1992.

BIBLIOGRAPHY

PUBLICATIONS (from 67 total)

1. Dean, P.N., Pinkel, D. and Mendelsohn, M.L. (1978). Hydrodynamic Orientation of Sperm Heads for Flow Cytometry. *Bio. Phys.* 23, 7-13.
2. Pinkel, D., Dean, P., Lake, S., Peters, D., Mendelsohn, M., Gray, J., Van Dilla, M.A. and Gledhill, B.L. (1979). Flow Cytometry of Mammalian Sperm: Progress in DNA and Morphology Measurement. *J. Histochem. Cytochem.* 27, No. 1, 353-358.
3. Van Dilla, M.A., Pinkel, D., Gledhill, B.L., Lake, S., Watchmaker, G., and Wyrobek, A.J. (1980). Flow Cytometry of Mammalian Sperm: Progress Report. Chapter IX in: *Flow Cytometry IV* (O.D. Laerum, T. Lindmo, and E. Thorud, Eds.), Universitetsforlaget, pp. 279-283.
4. Gledhill, B.L., Pinkel, D., Garner, D.L., and Van Dilla, M.A. (1982). Identifying X- and Y-Chromosome-Bearing Sperm by DNA Content – Retrospective Perspectives and Prospective Opinions. In: *Proc. of Prospects for Sexing Mammalian Sperm*, Denver, CO, (R.P. Amann and G.E. Sudel, Jr., Eds.), Colorado Univ. Press, Boulder, CO, pp. 177-191.
5. Pinkel, D., Lake, S., Gledhill, B.L., Van Dilla, M.A., Stephenson, D., and Watchmaker, G. (1982). High Resolution DNA Content Measurements of Mammalian Sperm. *Cytometry* 3, 1-9.
6. Pinkel, D., Gledhill, B.L., Lake, S., Stephenson, D., and Van Dilla, M.A. (1982). Toward Sex Pre-Selection in Mammals? Separation of Y and "#O" Chromosome-Bearing Sperm in the Vole *Microtus oregoni*. *Science* 218, 904-906.
7. Garner, D.L., Gledhill, B.L., Pinkel, D., Lake, S., Stephenson, D., Van Dilla, M.A., and Johnson, L.A. (1983). Quantification of the X- and Y-Chromosome- Bearing Spermatozoa of Domestic Animals. *Biology of Reproduction* 28, 312-321.
8. Pinkel, D., Gledhill, B.L., Van Dilla, M.A., Lake, S., and Wyrobek, A.J. (1983). Radiation-Induced DNA Content Variability in Mouse Sperm. *Radiat. Res.* 95, 550-565.
9. Pinkel, D. (1984). Cytometric Analysis of Mammalian Sperm for Induced Morphologic and DNA Content Errors. In: *Biological Dosimetry: Cytometric Approaches to Mammalian Systems* (Eisert, W.G. and Mendelsohn, M.L., Eds.), Springer-Verlag, pp. 111-126.
10. Garner, D.L., Johnson, L.A., Lake, S., Chaney, N., Stephenson, D., Pinkel, D., and Gledhill, B.L. (1985). Morphological and Ultrastructural Characterization of Mammalian Spermatozoa Processed for Flow Cytometric DNA Analyses. *Gamete Res.* 10, 339-351.
11. Pinkel, D., Garner, D.L., Gledhill, B.L., Lake, S., Stephenson, D., and Johnson, L.A. (1985). Flow Cytometric Determination of the Proportions of X- and Y-Chromosome-Bearing Sperm in Samples of Purportedly Separated Bull Sperm. *J. Animal Sci.* 60, 1303-1307.
12. Garner, D.L., Pinkel, D., Johnson, L.A., and Pace, M.M. (1986). Assessment of Spermatozoal Function Using Dual Fluorescent Staining and Flow Cytometric Analyses. *Biology of Reproduction* 34, 127-138.

13. Libbus, B.L., Perreault, S.D., Johnson, L.A., and Pinkel, D. (1986). Incidence of Chromosome Aberrations in Mammalian Sperm Stained with Hoechst 33342 and UV-Laser-Irradiated During Flow Sorting. *Mutation Research* 182, 265-274.
14. Gledhill, B.L., Evenson, D.P., and Pinkel, D. (1990). Flow Cytometry and Sorting of Sperm and Male Germ Cells. In: *Flow Cytometry and Sorting*, (M.R. Melamed, T. Lindmo and M.L. Mendelsohn, Eds.), Alan R. Liss, Inc., New York, 1990.
15. Wyrobek, A.J., Alhorn, T., Balhorn, R., Stanker, L., and Pinkel, D. (1990) Fluorescence In Situ Hybridization to Y-Chromosomes in Decondensed Human Sperm Nuclei. *Molecular Reproduction and Development* 27, 200-208.